ACID AND ALKALINE PHOSPHATASE CHANGES ASSOCIATED WITH FEEDING, STARVATION AND REGENERATION IN PLANARIANS[1]

PAUL J. OSBORNE AND A. T. MILLER, JR.

Department of Biology, Lynchburg College, Lynchburg, Virginia, and Department of Physiology, University of North Carolina, Chapel Hill, N. C.

The acid phosphatase activity in planarian gastrodermal cells has been reported to increase almost immediately following the ingestion of food (Rosenbaum and Rolon, 1960; Jennings, 1962b). Acid phosphatase has also been implicated in the early stages of digestion in the food vacuoles of paramecia (Müller and Törö, 1962) and of amebae (Müller, Toth and Törö, 1962). These results suggest an analogy between the food vacuoles of lower animals and the lysosomes (de Duve, 1961) of higher animals. Lysosomal enzymes are thought to be involved not only in the digestion of exogenous materials but also in the autolytic degradation of tissues in such processes as developmental involution and metamorphosis. It seems likely, therefore, that acid hydrolases may play a similar role in the gradual disappearance of digestive and reproductive organs during prolonged starvation in planarians, as well as in the provision of raw materials for the early stages of regeneration following transection, before the ingestion of exogenous food stuffs can be resumed.

Less is known about the significance of alkaline phosphatase than of acid phosphatase, although its frequent localization in absorptive epithelia in higher forms is suggestive of a role in the phosphorylation of certain compounds prior to their transport across membranes. High levels of alkaline phosphatase activity have been observed in several organs of planarians, including protonephridia (Danielli and Pantin, 1950), resting neoblasts (Pedersen, 1959) and nervous and muscular tissues (Gazso, Török and Rappay, 1961). Jennings (1962b) has recently reported the appearance of alkaline phosphatase in planarian gastrodermal cells several days following food ingestion, and he suggests that it may be concerned with the release of energy needed for secretion of the various digestive enzymes and for the absorption of the products of digestion from the food vacuoles.

The object of the present work was to extend the above-mentioned observations to later stages of digestion as they are succeeded by starvation effects, and to the tissue reconstruction involved in regeneration following transection.

Materials and Methods

All observations were made on specimens of *Dugesia tigrina* obtained from the Carolina Biological Supply Company. Frozen sections were used instead of paraf-

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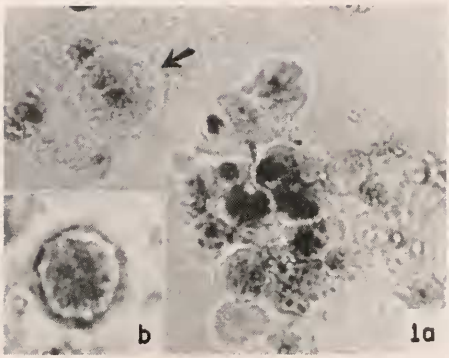
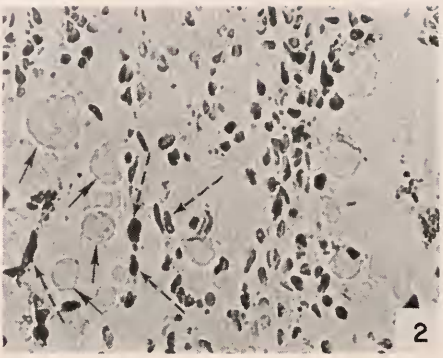
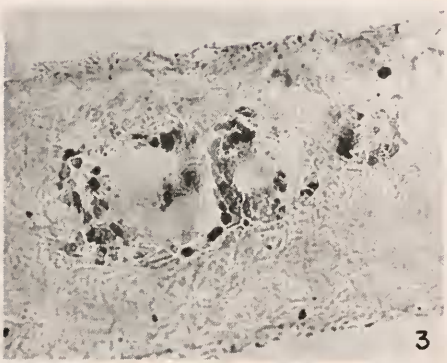
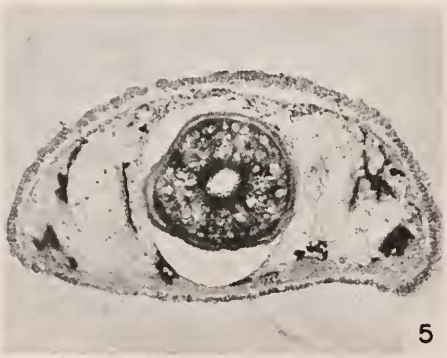








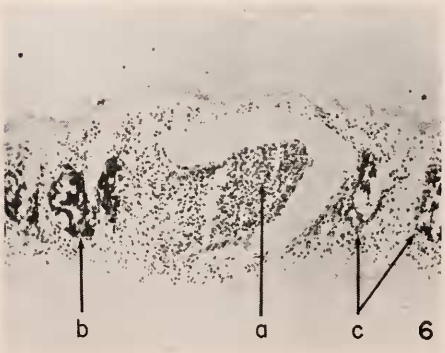


FIGURE 1a. Acid phosphatase activity in gastrodermal cells 24 hours after ingestion of cooked egg yolk. Arrow points to a gastrodermal cell containing two food vacuoles. 900 ×.

FIGURE 1b. Target form of gastrodermal cell, with acid phosphatase activity in cell membrane and in central core, two days after ingestion of cooked egg yolk. 1350 ×.

FIGURE 2. Eight days after ingestion of cooked egg yolk. The gastrodermal cells (solid arrows) are free of acid phosphatase activity and the gut region is being invaded by neoblasts (dotted arrows) with high acid phosphatase activity. 675 ×.

FIGURE 3. Thirty days after ingestion of cooked egg yolk. The intestinal epithelium is densely infiltrated by neoblasts with moderately high acid phosphatase activity. 300 ×.

FIGURE 4. Alkaline phosphatase activity in food vacuoles 24 hours after ingestion of cooked egg yolk. 300 ×.

fin-embedded sections, despite the better cytological preservation of the latter, because of the desire to preserve enzyme activity. This is especially important in the case of acid phosphatase, which undergoes considerable inactivation even when low-melting-point paraffin is used.

After a ten-day period of starvation (to eliminate the enzyme changes associated with previous feeding) the worms were allowed to feed on cooked egg yolk until satiated, and were then removed to fresh medium containing no food. At varying intervals after feeding, worms were slowly chilled to 4° C. and then fixed for 12–24 hours at 4° C. in 10% neutral formalin containing 1% calcium chloride. The fixed specimens were rinsed for 1–2 hours in distilled water at 4° C., then blotted and embedded in 10% gelatin, in the following manner. Melted gelatin was layered in the bottom of a small beaker and chilled until firm. Specimens were placed on top of the solidified gelatin, covered with a layer of melted gelatin and the beaker placed in a freezer until the gelatin had solidified. The gelatin was then cut into blocks, each containing a single worm; the blocks were mounted on cryostat object holders and frozen with dry ice or Freon. Sections were cut at 8 microns in a Pearse cryostat, mounted on chilled slides and air-dried. Acid and alkaline phosphatase activities were visualized by the methods of Gomori (1952).

For the studies on phosphatase changes during regeneration, planarians were starved for 10 days, the pharynx was carefully removed with dissecting needles, and the worm was cut transversely into equal halves which were allowed to regenerate in food-free medium. Each half regenerated its missing structures, including a pharynx, and the results to be described were identical in organisms regenerating from anterior and posterior halves.

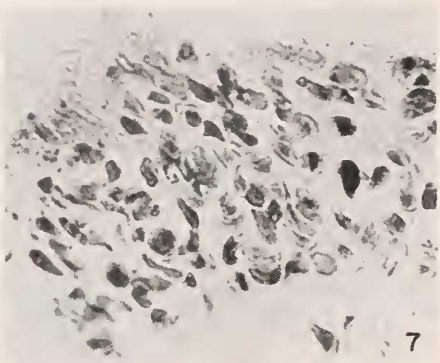
Results

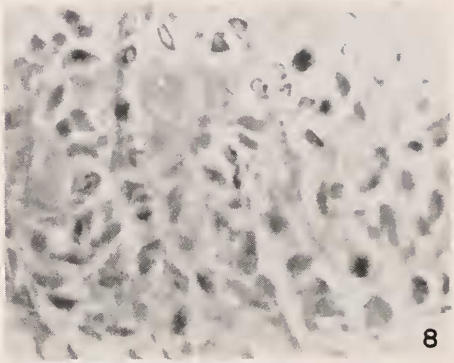
Twenty-four hours after the ingestion of cooked egg yolk there was a moderately high level of acid phosphatase activity in the gastrodermal cells (Fig. 1a). This confirms the reports of Rosenbaum and Rolon (1960) and of Jennings (1962b). The enzyme reaction product at this time was in the form of small granules which gradually increased in size and numbers until they filled the entire cell by the second day after ingestion of food. At this time some of the gastrodermal cells had a "target" appearance, with intense acid phosphatase activity in the cell membrane, separated by a clear zone from the central core of enzyme activity (Fig. 1b).

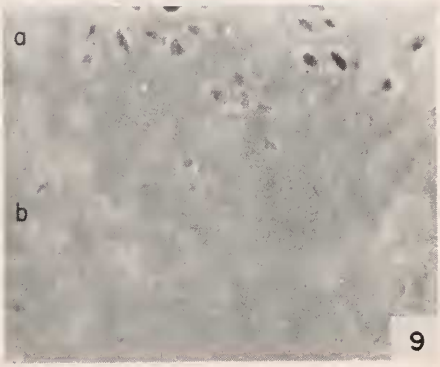
Acid phosphatase activity in the gastrodermal cells diminished rapidly after the second day following food ingestion; by the fourth day many of the cells had no demonstrable activity, though minimal activity persisted in a few cells up to seven days. Coincident with the decline in acid phosphatase activity in the gastrodermal cells there was a striking increase in the activity of this enzyme in the neoblasts. By the eighth day there was a definite invasion of the gut region by acid phosphatase-rich neoblasts (Fig. 2). Thirty days after the ingestion of food the walls of

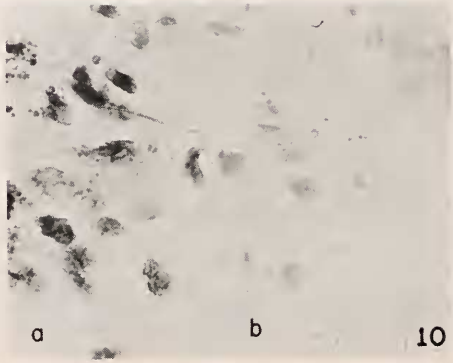
Figure 5. Alkaline phosphatase activity in pharynx, nerve fibers and glands after 60 days' starvation. 115 ×.

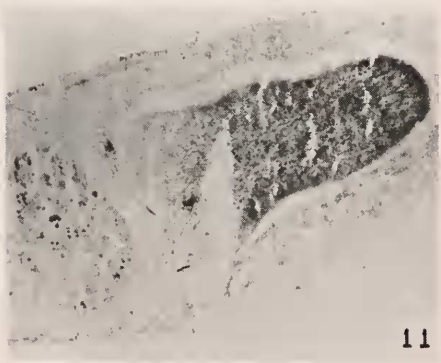
Figure 6. Regenerating planarian 6 days after transection (longitudinal section). The regenerating pharynx (a) is densely infiltrated with neoblasts having acid phosphatase activity, as are the original gut (b) and the regenerating gut (c). 75 ×.











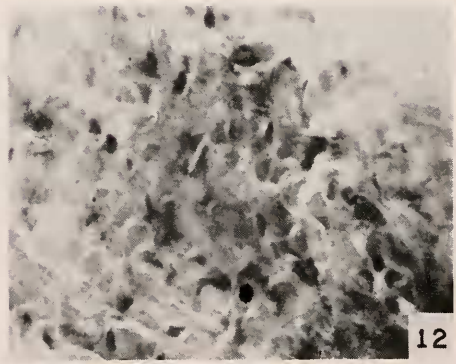


FIGURE 7. Higher magnification of cells of regenerating pharynx in Figure 6, showing neoblasts with acid phosphatase activity. 675 ×.

FIGURE 8. Phase contrast photograph of neoblasts in "inactive" region; edge of zone of regenerating gut at top of field. 675 ×.

FIGURE 9. Light microscope photograph of same field as Figure 8. Note the absence of acid phosphatase activity in all the neoblasts except those near the "active" region. a, zone of regenerating gut; b, inactive zone. 675 ×.

FIGURE 10. Gradient of acid phosphatase activity in neoblasts migrating (from right to left) to region of regenerating gut. a and b as in Figure 9. 675 ×.

the gut diverticuli were densely infiltrated by neoblasts in which the level of acid phosphatase activity was beginning to decline (Fig. 3). Coincident with the changes in the gut region, high levels of acid phosphatase activity were observed in the mucous glands and ventrolateral slime tracts and in the neoblasts in the pharynx.

The ingestion of cooked food was followed by the appearance of alkaline phosphatase activity in the forming food vacuoles (Fig. 4). After reaching a peak one to two days after food ingestion, the enzyme activity diminished somewhat more slowly than did that of acid phosphatase and was absent by the eighth day. Alkaline phosphatase activity in nerve fibers, mucous glands and pharynx was not affected by feeding and was undiminished after periods of starvation as long as 60 days (Fig. 5).

Preliminary studies have been made on phosphatase changes associated with regeneration following transection. On the sixth day after transection the regenerating pharynx was heavily infiltrated with neoblasts which had a moderately high level of acid phosphatase activity (Figs. 6 and 7). Figure 6 also shows an infiltration of both the original and the regenerating gut with neoblasts rich in acid phosphatase, suggesting that these cells were participating both in the degradation of the original gut (starvation effect) and in the reconstitution of the gut in the regenerating portion of the worm. The neoblasts at some distance from the sites of organ destruction or reconstitution ("resting" neoblasts) were lacking in acid phosphatase activity. As they migrated toward these sites they appeared to acquire progressively increasing amounts of acid phosphatase (Figs. 8–10).

The regenerating pharynx was also rich in alkaline phosphatase, predominantly localized in the neoblasts (Figs. 11 and 12).

Discussion

The changes in acid and alkaline phosphatase activities associated with feeding, starvation and regeneration in planarians suggest that these enzymes are intimately involved in the processes of nutrition, growth and repair. Little is known, however, about the stimuli to enzyme induction and the exact chemical reactions which are catalyzed by these enzymes *in vivo*.

Rosenbaum and Rolon (1960) reported the appearance of acid phosphatase activity in the gastrodermal cells of *Dugesia dorotocephala* and *Dugesia tigrina* within five minutes after the feeding of cooked liver, and they identified this with the formation of food vacuoles. Maximum acid phosphatase activity was present in all the food vacuoles 24 to 48 hours after feeding; the level of enzyme activity began to decline three days after feeding and was absent one week after feeding. Similar changes were observed in aminopeptidase and β-glucuronidase activities, although intracellular localization of the latter enzyme was not achieved. Müller, Toth and Törö (1962) described a similar relation of acid phosphatase and nonspecific esterase activity to the food vacuole cycle in *Amoeba proteus*. In unfed amebae, fine granules giving an intense acid phosphatase reaction (lysosomes?)

Figure 11. Regenerating pharynx 6 days after transection. The infiltrating neoblasts are rich in alkaline phosphatase. 75 ×.

Figure 12. Higher magnification of regenerating pharynx in Figure 11, showing alkaline phosphatase in neoblasts. 675 ×.

were distributed at random. Recently-formed food vacuoles showed only moderate enzyme activity but strongly staining granules were observed around these early vacuoles, often forming large aggregates. The authors suggest that these granules may be "enzyme carriers." Vacuoles containing still living or dying prey (*Tetrahymena pyriformis*) showed no demonstrable increase in acid phosphatase activity ("ingestion vacuoles"), but a sharp rise in enzyme activity occurred when the food organisms had been killed and most of the water had disappeared from the vacuoles ("digestion vacuoles"). Parallel changes occurred in non-specific esterase activity. The authors relate the increased activities of the two enzymes to the processes of intracellular digestion of food and suggest that ameba digestion vacuoles can be considered as lysosomes. Presumably they would be representatives of the type called "lysophagosomes" by de Duve (1961).

Our results confirm the recent report of Jennings (1962b) of a sequential appearance of acid and alkaline phosphatase activities in the gastrodermal cells of planarians following food ingestion. The apparently greater intensity and longer persistence of acid phosphatase activity in our preparations is probably due to better enzyme preservation in frozen sections than in paraffin-embedded material. There is general agreement that acid phosphatase and other acid hydrolases are involved in the initial stages of digestion in food vacuoles (Rosenbaum and Rolon, 1960; Müller *et al.,* 1962; Jennings, 1962b) but the function of the alkaline phosphatase which appears some hours after the beginning of digestion is unknown. Jennings (1962b) suggests that it "may be concerned in the release of energy needed for secretion of the various enzymes and the absorption of the products of digestion from the vacuoles." In studies on the rhynchocoelan, *Lineus ruber,* Jennings (1962a) reported the appearance of intense alkaline phosphatase activity in the luminal margins of the gut cells immediately after feeding, and he postulated a role of the enzyme in the process of phagocytosis of food. No such immediate response of alkaline phosphatase to food ingestion has been observed in planarians.

Prolonged starvation in planarians is characterized by the resorption of certain structures, notably the digestive and reproductive systems, and by the persistence of other more immediately essential structures, such as the pharynx, nervous system, protonephridia and lateral mucous glands. Survival for periods in excess of 60 days without food has been observed, and interesting changes in phosphatase activity occur during starvation. The earliest of these changes is a striking increase in acid phosphatase activity in the neoblasts, especially those surrounding the gut region, which is clearly in evidence on the fourth day following feeding. In the succeeding days the neoblasts infiltrate the gut where their hydrolytic enzymes presumably participate in the autolytic degradation of the gastrodermal cells in the later stages of starvation.

The sequence of changes in alkaline phosphatase activity during starvation is quite different. Following the decline in activity in the gastrodermal cells, leading to disappearance of the enzyme about eight days following feeding, there is no recurrence as in the case of acid phosphatase. However, the alkaline phosphatase activity of those structures which do not undergo resorption during starvation seems to be unaffected by feeding and starvation. Thus, even after 60 days starvation, there is intense alkaline phosphatase activity in the pharynx, the nerve fibers and the lateral mucous glands, *i.e.,* those structures which by preserving the

capacity for locomotion and for ingestion of food, favor the survival of the organism.

The preliminary observations on regenerating planarians suggest that the neoblasts play a role both in the (probably) degenerative changes in the gut of the original segment and in the reconstitution of organs in the regenerating segment. In both cases the neoblasts in these sites have a high level of acid phosphatase activity. Since the "resting" neoblasts show no acid phosphatase activity (Figs. 8–10), this must represent enzyme induction and a participation of acid phosphatase in both degradative and regenerative processes. The neoblasts in the sites of organ regeneration have intense alkaline phosphatase activity also, but the functional significance of this observation is uncertain, since resting neoblasts also show alkaline phosphatase activity (Pedersen, 1959).

The results of the present study implicate acid phosphatase in a variety of degradative processes, presumably autolytic in nature, all of which results in the conversion of endogenous or exogenous compounds into building materials for maintenance and repair. An apparent discrepancy between our results and those of earlier studies on the histological changes during starvation in planarians requires some comment. Willier *et al.* (1925) reported that the cells of the intestinal epithelium show practically no change until after six weeks of starvation, when they begin to undergo degeneration with reduction in size. Our observation of intense acid phosphatase activity in the region of the intestinal epithelial cells after much shorter periods of starvation suggests that the enzymatic phase of autolytic degradation may begin much earlier, but that this does not lead immediately to histologically demonstrable degeneration of the cells. The participation of acid phosphatase in the reconstitution of organs in regenerating planarians suggests the simultaneous occurrence of degradative and synthetic reactions, the one perhaps providing the raw materials for the other. The functional significance of the alkaline phosphatase changes is unknown. Its presence in the neoblasts in regions of regeneration may reflect a participation in organogenesis (Junqueira, 1950; Vorbrodt, 1958), but this is conjectural in view of the occurrence of alkaline phosphatase in resting neoblasts.

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Summary

1. The ingestion of cooked food by starved planarians is followed by the appearance of high levels of acid, and later of alkaline phosphatase activities in the gastrodermal cells. The enzyme activities decline gradually, to disappear after 7–10 days. Beginning about 4 days after feeding, the gut region is progressively invaded by neoblasts with high acid phosphatase activity. Intense alkaline phosphatase activity persists in certain "essential" structures (nerve fibers, gland cells, protonephridia) even after periods of starvation as long as 60 days.

2. During regeneration following transection, neoblasts rich in acid phosphatase invade both the regenerating organs and the degenerating structures of the original

segment. This represents enzyme induction, since resting neoblasts show no acid phosphatase activity. Neoblasts with alkaline phosphatase activity are abundant in the regions of regeneration, but the significance of this observation is uncertain since alkaline phosphatase activity also characterizes resting neoblasts.

3. It is suggested that the lysosomal acid hydrolases (typified by acid phosphatase) are involved not only in the early stages of digestion in the food vacuoles, but also in the autolysis of dispensable organs during starvation and in the tissue breakdown which precedes regeneration in transected planarians.

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